

FUNDAMENTAL STUDIES ON THE ELECTRICAL POTENTIAL DIFFERENCE ACROSS BLOOD VESSEL WALLS AND APPLICATIONS OF DIRECT CURRENT COAGULATION

HARUO HAYASHI

*1st Department of Surgery, Nagoya University School of Medicine
(Director: Prof. Yoshio Hashimoto)*

ABSTRACT

In recent years, the improvement in surgical techniques have been greatly improved with the development of medical electronics. And yet, there remains many incidences in which the surgeon should use the classical techniques rather than the recently developed procedures. For example, when oozing or uncontrollable bleeding is encountered from injured organ in which ligation or suturing, application of pressure, or application of alternating electro-coagulation may be used, but no satisfactory result may be obtained with any of these methods.

In this regard, the effect of the direct current on the living body has been studied by many investigators since the middle of the 18th century. Recently, the application of the direct current electro-coagulation has been applied clinically by some investigators. This electric hemostasis is merely a skillful application of the bio-electric phenomena to living body. In these experiments, the investigation was carried out on the bio-electric phenomena, especially on the potential difference of the blood vessel wall which previously had been studied in the various aspects by many investigators. However, different interfering factors obstruct the measuring of potential difference across the blood vessel wall under the normal condition.

This paper describes the new apparatus for measuring of potential difference of the removed blood vessel wall using a special electrode which is not affected by the phenomena of polarization. Intravascular thrombus formation due to direct current was observed by the microscope and microphotographs were taken.

In animal experiments, stable injury on the organ (spleen), electro-coagulation was done under various conditions for oozing or uncontrollable bleeding and its effect on the injured portion was investigated. At the same time, blood pressure, platelets, and hematocrit (factors that are concerned in clotting mechanism) which is changed due to direct current, were measured, and the results were discussed.

INTRODUCTION

In 1824, the first person who reported *in vitro* blood clotting produced by

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Received for publication August 22, 1967.

electric current was Scudamore¹⁾. He observed that blood precipitated on the positive electrode but not on the negative electrode. Next, in 1895, the possibility that blood clotting could be catalyzed by an electric current was first suggested in an article written by Poore²⁾ in "Quain's Medical Dictionary". Thereafter, the effect of electric current on living body was not attempted until the beginning of the 20th century.

In 1916, Stern³⁾ studied the relationship between the strength of electric current and the metallic electrode necessary for blood coagulation by the method of Cannon and Mendenhall, "Factors affecting the coagulation time of blood" in which it was claimed that aluminum used as positive electrode was better than other materials, and aluminum would reduce the clotting time about 90 per cent. However, platinum electrode has not been used at this time.

Afterward, the electrophoretic characteristics of mammalian blood cells were reported by Abramson⁴⁾⁵⁾⁶⁾. In 1924, he reported that injury was one of the forces which moved white blood cells, to the point of injury because the injured point was usually positively charged. The superiority of platinum electrode over copper or aluminum has been recognized since that time.

In 1936, Burge *et al.*⁷⁾, reported an electrical current between injured and uninjured segments of carotid artery. The injured segment was negatively charged in respect to the intact portion.

In 1953, Sawyer *et al.*⁸⁾⁹⁾¹⁰⁾¹¹⁾, studied this problem of direct current coagulation very extensively and reported many experimental results. Afterward, they applied it to clinical cases with good results, but this method has not been widely adapted.

Sawyer and Pate investigated the fundamental effect of direct current on living bodies and demonstrated in 1953, that the blood vessel wall has a negative potential difference with the intima in respect to the adventitia. The magnitude of the potential difference is - 3 to - 15 mV in the normal blood vessel, and gross injury such as transection, crushing, and rough dissection usually caused at least local reversal of the potential difference; that is, the intima became positive to the adventitia. And they said that this was due probably to volume conduction between the injured and uninjured vessels and between vessels and the surrounding tissues. Thus, the injury current may create an electrophoretic force which attracted and held negatively charged platelets, red blood cells, white blood cells, and proteins against the injured wall. These bio-electric phenomena may constitute one of the etiological factors in thrombus formation, and the Abramson's hypothesis in 1924 was supported by these studies.

In the same year, Sawyer and co-workers reported on studies concerning the potential difference in the implantation of the fresh and freeze-dried grafts.

In 1955, Sawyer¹²⁾¹³⁾ showed again the relation of bio-electric phenomena

and thrombus formation and emphasized that if a normal blood vessel is placed in an electric field as low as 1 to 2 mV, thrombosis will form on the positively charged wall and not on the negatively charged wall opposite it.

Recent studies demonstrated that the negative charge of an injured blood vessel could delay intravascular thrombus formation by using a high impedance voltmeter in series with a pair of measuring electrode. Attempts were made to measure potential difference along and across the wall of the abdominal aorta of dogs by Eisner¹⁵⁾ in 1957.

Sawyer¹⁴⁾ reported generally about potential difference across the wall of vessel in 1962, as follows: Several types of experiments characterizing the electrical properties of blood vessels have been carried out over the past decade. As the basic subject, it became known that the normal state of potential difference between the adventitia and the intima, or the blood within the vessel, was demonstrated to be 1 to 5 mV with the blood vessel wall or the intima relatively negative in respect to the adventitia. Injury altered normal potential difference and when the polarity was reversed, making the blood or the intima relatively positive in respect to the adventitia, thrombosis frequently occurred, and a change of potential difference was influenced by the surrounding tissues of the blood vessel.

In recent studies, Sawyer¹⁷⁾ demonstrated that *in vitro* experiments have been developed to measure the transmural potential difference across the membrane of the aortic wall.

In 1959, Schwartz¹⁸⁾ described his experimental results that thrombi formed at the end of the 1 hour experimental period in all cases which received 4.5 V, 5 mA currents with platinum positive electrode surrounding the femoral veins of dogs. The microscopic examination revealed no destruction of the blood vessel wall, and histologically the thrombi were identical to spontaneous thrombi. And he performed experiments in relation of electrical thrombosis to dicumarol or heparin.

In 4 control limbs with the same mechanical preparation but without the application of a positive current, no thrombi formed during the 1 hour experimental period. In the 4 dogs given dicumarol, the prothrombin time was reduced to less than 30 per cent of the normal control as measured by a modified one stab test. The superficial femoral vein was then subjected to a 5 mA positive current as previously described. At the end of 1 hour, the vein was opened and inspected for thrombi. In each of the 4 vessels studied, significantly large thrombi were found.

4 animals received aqueous heparin in sufficient quantities to increase the clotting time, as performed by the Lee-White technique, to over 30 minutes, and then 5 mA of positive current were imposed upon the superficial femoral vein in a similar fashion. After a 1 hour experimental period, the vein were opened and in none of the animals was a thrombus evident.

Sawyer¹⁹⁾ observed the process of electrical intravascular thrombus formation and alteration of potential difference which occurred in capillaries of the mesentery of rat (1960). According to his report, microscopic observation indicated that precipitation of cells, ostensibly the beginning of thrombus formation, first occurred on the blood vessel wall nearer the positive electrodes, and many small free emboli were noted during the experimental procedure.

In 1961, at last, Sawyer^{20) 21) 22)} attempted the clinical application of electro-coagulation to bleeding in the injured portion. In animal experiments, the effect of the electro-coagulation technique was tested in rats or dogs by determining its result in hemostasis in the hemitranssected liver and incisions into the one of the spleens or kidneys. Furthermore, he demonstrated that the function of the kidney was normal after electro-coagulation. In clinical trial, the direct current coagulation technique has been compared with alternating current electro-coagulation technique in the operating room to determine their comparative effectiveness and consequential comparative tissue destruction. He reported that direct current coagulation is not as rapid as the method of alternating current electro-coagulation when applied to small "bleeders". But he demonstrated that direct coagulation is effective in causing hemostasis in diffusely bleeding surfaces but less effective with blood vessel of high pressure greater than 1 to 2 mm in diameter.

Examinations of the animal tissues through which the current has been passed showed little histological evidence of tissue damage. Only cell directly beneath the positive electrode, to a depth of 4 to 5 cell layers, appeared crenated. No other morphologic evidence of damage could be seen. The blood which collected beneath the positive electrode demonstrated histologic evidence of clotting.

Sawyer demonstrated that application of the above described technique to be selectively useful in controlling the oozing from raw surfaces of liver, spleen, kidney, pleura, and prostate and that currents of up to 100 mA have been passed in the living body without visible unfavorable results.

In 1964, Kravitz²⁴⁾, a plastic surgeon, tried direct current coagulation for uncontrollable bleeding by transistorized Heathkit Model IP-20, and he reported that a current of 12 to 16 mA used for a period of 7 to 10 minutes is usually sufficient to produce coagulation on diffuse bleeding surfaces, but the blood vessels greater than 1 to 2 mm in diameter, however, must be ligated. He said also that as much as 100 mA, has been used on the experimental animal (living body) without unfavorable effect. His studies, histologic section in dogs, taken from bleeding surfaces under the positive electrode before and after the application of electro-coagulation using (less than 25 mA), showed no significant differences.

MATERIALS AND METHODS

Experiment (1)

As described before, method of potential difference measurement of the blood vessel wall used since 1951 seems to be extremely unreliable, because the measurement of potential difference was affected by so many factors and was done under unfavorable conditions. Therefore, the measurement was done *in vitro* with the removed blood vessels in all cases.

All experiments are carried out on adult mongrel dogs anesthetized with intraperitoneal sodium pentobarbital (Isozol) of 0.25 to 0.35 g. A mid-line abdominal incision was made, and a sectional abdominal aorta 1 to 1.5 cm in diameter and 3.5 to 6 cm long was removed, then it was placed between induced tube A and B immediately.

Next, induced tubes and removed blood vessel were placed in 4 liters glass vessel. Two comparative electrodes (Model HC-205) placed in the glass vessel were connected with the micro volt-ammeter (Model PM-18); one as a positive, and the other as a negative electrode. In addition, the micro volt-ammeter was connected with poly-recorder to record accurate potential difference (Figs. 1, 2, 3 and 4). And then, the glass vessel and the induced tubes were filled up with 1 per cent KCl solution (pH 5.72).

The negative electrode was placed in

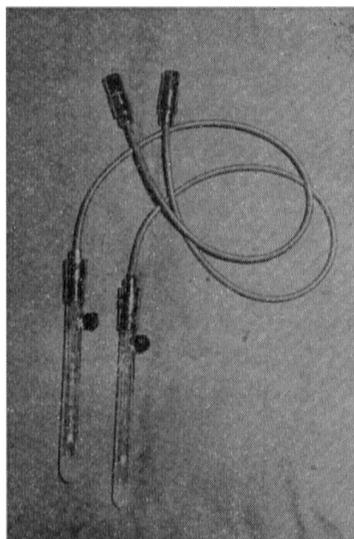


FIG. 1. Comparative electrode (Model HC-205).

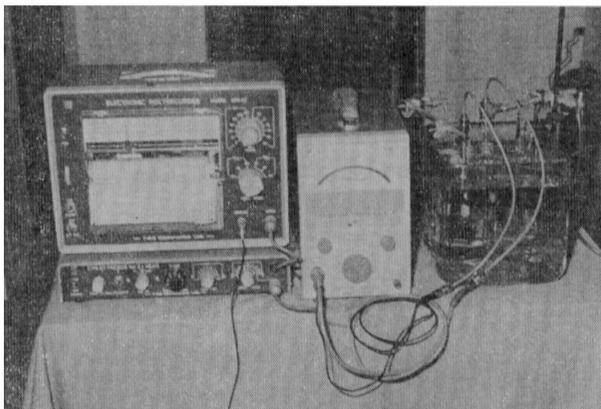


FIG. 2. Photograph of the new apparatus of p.d. measurement of the removed blood vessel.



FIG. 3

FIG. 3. Micro volt-ammeter (Model PM-18), fully assembled.

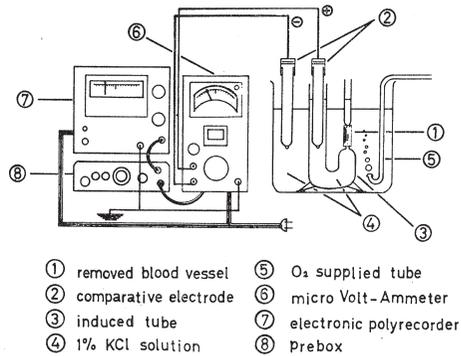


FIG. 4

FIG. 4. Schematic diagram of the new apparatus of p.d. measurement of the removed blood vessel.

the glass vessel and potential difference was measured by reading the amplitude of the indicator of micro volt ammeter. When positive electrode entered into the induced tube A and took out from one.

In order to solve the question that potential difference measured by this apparatus might be diffused electric potential between the removed blood vessel wall and 1 per cent KCl solution, the following pilot experiments were performed successively.

1) The glass vessel and A and B induced tubes were filled with 1 per cent KCl solution, and rubber tube was placed between both induced tubes instead of the removed blood vessel.

2) Abdominal aorta, which had been soaked in formalin (10 per cent) for 4 to 24 hours, was placed be-

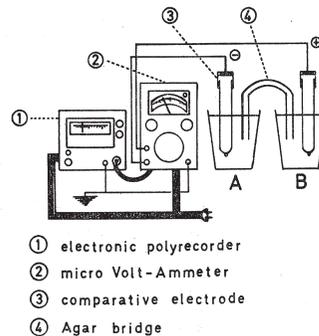


FIG. 5. Schematic diagram of the preliminary experiments:

A and B glass vessels were filled with different solution, and connected with agar bridge. A comparative electrode was put in each solution; one was charged positively and the other charged negatively.

They were connected with micro volt-ammeter and polyrecorder, and so, p.d. between both solutions were measured.

tween induced tubes instead of fresh removed blood vessel.

3) The potential difference was measured between 1 per cent KCl solution and one of the following materials through an agar bridge (Fig. 5).

1 per cent KCl solution: 1 per cent KCl solution

1 per cent KCl solution: dog blood

1 per cent KCl solution: distilled water

1 per cent KCl solution: Dextran

distilled water: distilled water

Furthermore, on account of measuring potential difference of the blood vessel wall under a condition as near as possible to the living body, the induced tubes were filled with heparinized dog blood instead of 1 per cent KCl solution.

Next, the removed blood vessel was injured by compression with a hemostat. The injured blood vessel was placed between induced tubes, and its potential difference was measured which was thought to be injured current.

Experiment (2)

The change in the capillary during the direct current coagulation was observed by microscope. Normal adult rabbits were used as experimental animals. They were anesthetized by intraperitoneal injections of 0.1 g sodium pentobarbital. A mid-line abdominal incision was made, the rabbit's mesentery was extracted and placed over the rubber plate, 15 by 10 by 1.5 cm in size with a transilluminated window.

The mesentery was soaked in normal saline solution at temperature of

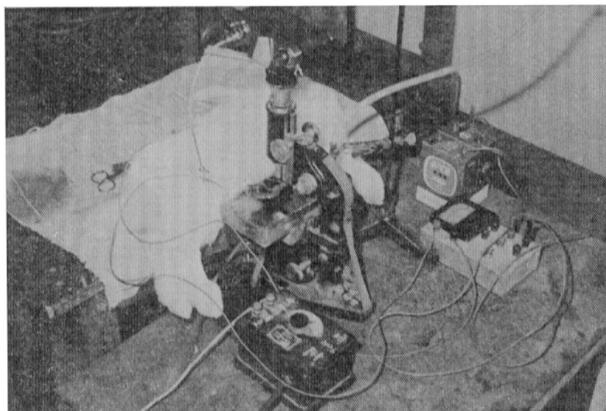


FIG. 6. The mesentery of rabbit was placed over the rubber-plate having a transilluminating window.

The circuit was made, and 3 or 6 V, 4 to 4.5 mA of positive current were flowed.

The process of electric intracapillary thrombus formation was observed microscopically through $\times 200$ objective lens and it was microphotographed at 30 seconds interval.

37°C. The positive platinum wire electrode, 0.1 mm in diameter, was placed over the mesentery, and negative electrode was buried in the femoral portion of the animal. Both electrode was connected to 3 or 6 V dry-cell battery to give positively charged electric field around the mesenteric capillary (Fig. 6).

During the application of a positive current, intracapillar changes were observed from time to time by means of a microscope of magnification up to 150 and photographed at 30 seconds intervals.

Experiment (3)

Based on the data from the Experiment (1) and Experiment (2), the effect of the direct current coagulation for uncontrollable bleeding from injured portion of animals was examined by the method similar to Sawyer (1961) and Kravitz (1964). And changes in blood pressure, platelets, and hematocrit occurring by the application of a positive current was observed.

All experiments are carried out on adult mongrel dogs. The animals were anesthetized by intraperitoneal injections of 0.25 to 0.35 g sodium pentobarbital (Isozol). A mid-line abdominal incision was made and the spleen was extracted out of the abdominal cavity.

A wound, 1 by 1.5 by 1 cm in size, was made on upper pole of the spleen by rongeur, and positive platinum plate electrode, 1.5 by 1 by 0.4 cm in size, was placed on the wound. Negative electrode was buried in the femoral muscle. Both electrodes were connected to dry-cell battery and ammeter to make a circuit (Fig. 7).

The experimental results were compared between the control group and groups with the application of a positive current (3, 6, 9 and 12 V), and each change of blood pressure, platelets, and hematocrit before and after the application of a positive current was observed.

Blood pressure was measured with a simple sphygmomanometer through a catheter inserted into the femoral artery exposed in advance. The blood samples for measurement of platelets and hematocrit were gathered from injured portion before and after the procedure. The platelets were counted using a phase contrast microscope by the Feissly and Ludin's method⁽²⁵⁾⁽²⁶⁾.

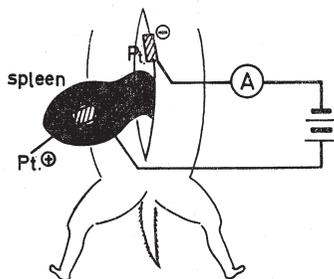


FIG. 7. The spleen of dog was taken out of the abdominal cavity, and a part of it was given the definite injury. The positive electrode (platinum plate) was placed over the injured portion, and negative was buried in other portion of dog.

The electro-coagulations were carried out at the voltages of 3, 6, 9 and 12 V.

RESULTS

(1) The potential difference across the removed blood vessel wall of dog's abdominal aorta, *in vitro*, under various conditions were seen in figures (Figs. 8, 9, 10, 11 and 12).

- 1) Potential difference of the normal blood vessel wall: 0.25 to 0.3 mV at 5 mV range (KCl:KCl)...(Fig. 8).
- 2) Potential difference of the normal blood vessel wall: 0.7 to 1 mV at 25 mV range (KCl:KCl)...(Fig. 9).
- 3) Potential difference of the normal blood vessel wall: 1.5 to 2 mV at 25 mV range (KCl:dog blood)...(Fig. 10).
- 4) Potential difference of the injured blood vessel wall: 0.25 to 0 mV at

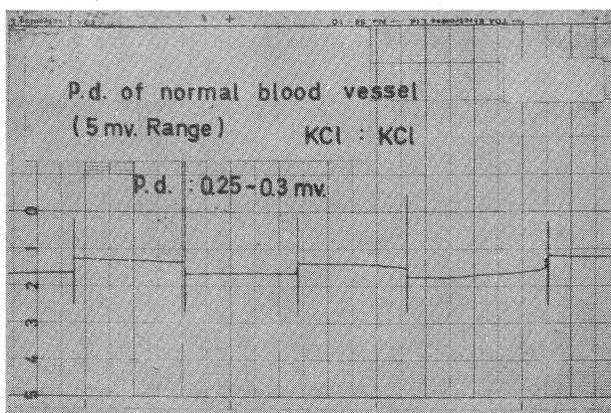


FIG. 8. Record indicated the p.d. across the normal removed blood vessel wall of dog, measured at 5 mV range. (KCl:KCl)

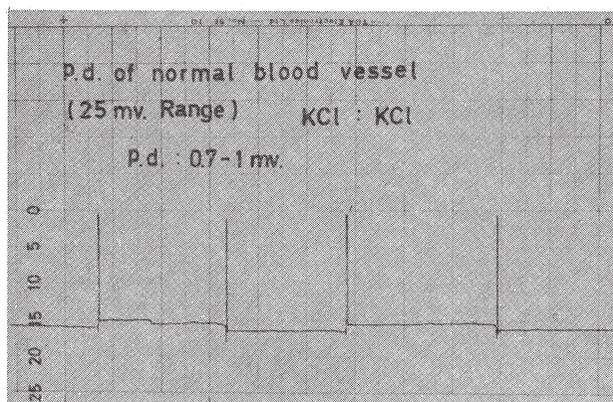


FIG. 9. Record indicated the p.d. across the normal removed blood vessel wall of dog, measured at 25 mV range. (KCl:KCl)

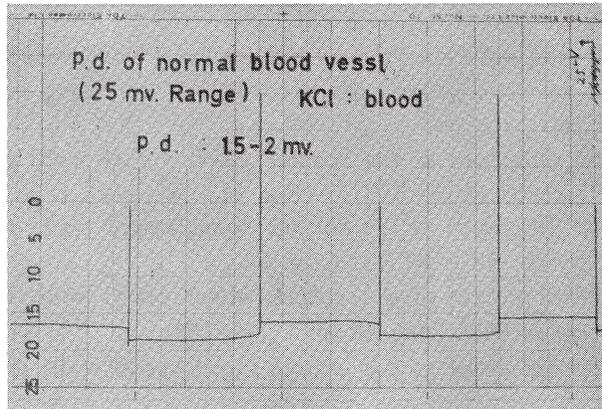


FIG. 10. Record indicated the p.d. across the normal removed blood vessel wall of dog, measured at 25 mV range. (KCl : dog blood)

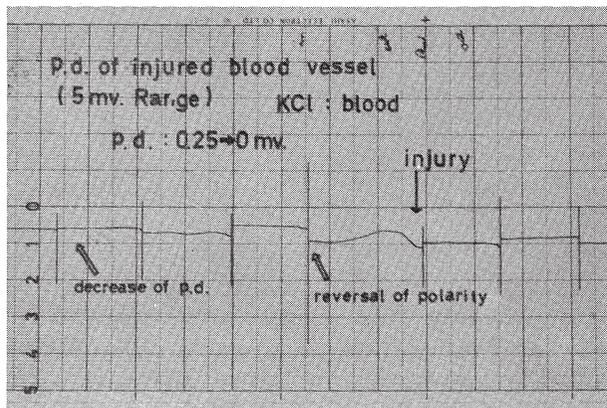


FIG. 11. Record indicated the p.d. across the injured removed blood vessel wall of dog, measured at 5 mV range. (KCl : dog blood)

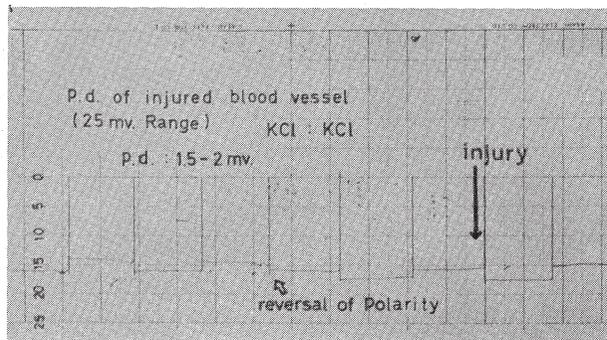


FIG. 12. Record indicated the p.d. across the injured removed blood vessel wall of dog, measured at 25 mV range. (KCl : KCl)

5 mV range (KCl:dog blood)...(Fig. 11).

5) Potential difference of the injured blood vessel wall: 1.5 to 2 mV at 25 mV range (KCl:KCl)...(Fig. 12).

These values were similar to Sawyer's report.

As shown in Figs. 11 and 12, potential difference decreased gradually, and a phenomenon of the local reversal of polarity was recorded. The two phenomena were thought to indicate the intravascular thrombus formation by the application of a positive current.

When the rubber tube was placed between the induced tube A and B instead of the removed blood vessel, the potential difference was -16 mV, and when the removed blood vessel preserved in formalin (10 per cent) for 4 to 24 hours was used, the potential difference was 0 mV (Fig. 13).

The potential difference between KCl and KCl, KCl and dog blood, KCl and distilled water, KCl and Dextran, and distilled water were -15, -19, -58, -22, and -45 mV, respectively (Fig. 14).

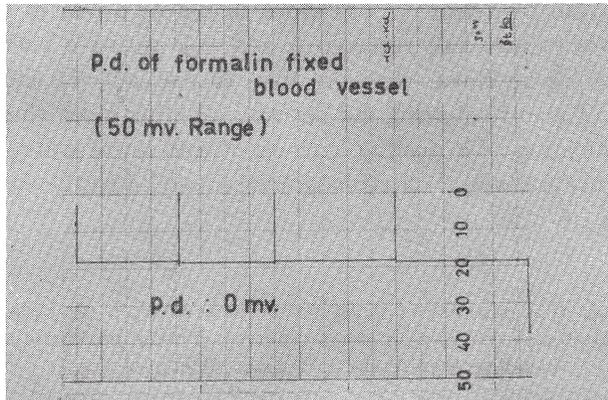


FIG. 13. Record showed the p.d. across the removed blood vessel which was soaked with 10 per cent formalin for 4 to 24 hours.

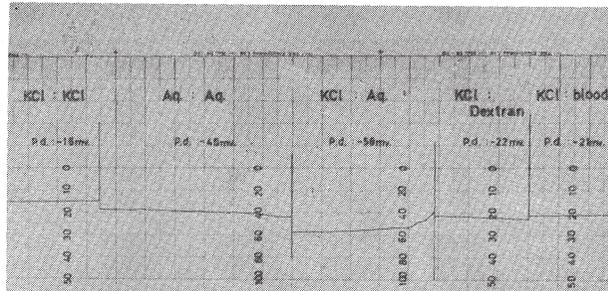


FIG. 14. Record of the p.d. between 1 per cent KCl solution and 1 per cent KCl solution, distilled water, dog blood, Dextran, and distilled water and distilled water.

(2) The following microscopical observations were obtained with the application of a positive current: Sludge phenomena of intracapillary occurred temporarily for 1 to 2 minutes after being received 6 V current, then disappeared (Photos. 2 and 3). When the direct current was increased to 4 to 4.5 mA, the sludge phenomena occurred in a great many of the least capillary of all visual field again at 6 to 7.5 minutes, and it was observed that the blood stream was slower (Photos. 4 and 5). And at last, the blood stream stopped completely (completion of thrombus formation) after the positive current was applied for 9 to 12 minutes (Photo. 6). And after 1 to 2 minutes of the thrombus formation, homogeneous situation which seemed to be irreversible, appeared in all capillaries (Photo. 7).

(3) The results of Experiment (3) were shown in Tables 1, 2, 3, 4 and 5. Bolod pressure decreased in more than 80 per cent of cases, after electro-coagulation (Table 6). Platelets decreased relatively compared with after electro-coagulation (Table 7). Hematocrit increased a little compared with before electro-coagulation (Table 8). However, the effect of electro-coagulation was a slight for bleeding from artery more than 1 to 2 mm in diameter.

In the groups which had received 6 and 9 V positive current, the most definite results were obtained. In more than 80 per cent of 3 and 12 V groups, the phenomena of electro-clotting did not appear after more than 25 minutes; that is, the application of a positive current of both lower voltage with weaker current and higher voltage with stronger current could on the contrary delayed the hemostasis.

TABLE 1. The changes of blood pressure, hematocrit, and platelets in non electro-coagulation group (control) for the uncontrollable bleeding from definitely injured spleen of dog.

Control							
	No.	1	2	3	4	5	Mean
	Sex	F	F	M	F	M	
	Weight	11.0 kg	8.0 kg	8.0 kg	8.5 kg	9.0 kg	
Blood pressure (mmHg)	b.	92	160	106	102	106	116
	e.	90	28	100	48	88	66
Ht. (%)	b.	29	46	10	42	37	32
	e.	32	42	29	48	40	38
Platelet	b.	84000	132500	126000	91500	131600	111000
	e.	66400	14900	9900	54000	104600	89000
Current	Q.	—	—	—	—	—	—
Coagulation	T.	12 min.	25 min. ↗				

TABLE 2. The changes of blood pressure, hematocrit, and platelets in electro-coagulation group (3 V) for the uncontrollable bleeding state.

3 V							
	No.	6	7	8	9	10	Mean
	Sex	M	F	M	F	F	
	Weight	10.0 kg	9.0 kg	7.0 kg	9.0 kg	7.5 kg	
Blood pressure (mmHg)	b.	130	110	82	118	104	110
	e.	—	102	80	122	86	102
Ht. (%)	b.	40	40	37	35	31	33
	e.	42	52	48	38	27	46
Platelet	b.	120000	—	195000	180500	184200	178000
	e.	108000	—	87000	141000	125800	136000
Current	Q.	5 mA	4 mA	3.5 mA	4 mA	3.5 mA	4 mA
Coagulation	T.	25 min. ↗	16 min.	6 min.	25 min. ↗	25 min. ↗	25 min. ↗

TABLE 3. The changes of blood pressure, hematocrit, and platelets in electro-coagulation group (6 V) for the uncontrollable bleeding state.

6 V							
	No.	11	12	13	14	15	Mean
	Sex	F	M	F	F	F	
	Weight	6.0 kg	9.0 kg	10.0 kg	8.0 kg	9.0 kg	
Blood pressure (mmHg)	b.	120	140	100	110	62	106
	e.	80	70	103	108	60	84
Ht. (%)	b.	22	25	39	30	45	31
	e.	23	29	35	41	50	34
Platelet	b.	—	195000	154600	156000	72500	141000
	e.	—	163000	136200	62000	76000	109000
Current	Q.	8 mA	9 mA	7 mA	7 mA	8 mA	8 mA
Coagulation	T.	13 min.	8 min.	10 min.	9 min.	19 min.	11 min.

In histological examination of the animal tissues through which the current has been passed, the picture of bleeding and congestion was recognized, and clotting is seen in the extravascular blood in all cases. Sinus and vascular

TABLE 4. The changes of blood pressure, hematocrit, and platelets in electro-coagulation group (9 V) for the uncontrollable bleeding state.

9 V		No.	16	17	18	19	20	Mean
	Sex	F	M	F	F	M		
	Weight	5.0 kg	7.5 kg	9.0 kg	9.0 kg	8.5 kg		
Blood pressure (mmHg)	b.	90	142	106	136	128	112	
	e.	86	164	122	96	110	118	
Ht. (%)	b.	35	44	27	22	33	32	
	e.	44	36	31	42	37	41	
Platelet	b.	127700	407000	61000	124800	104000	117000	
	e.	94600	—	111000	109900	98800	105000	
Current	Q.	17 mA	20 mA	19 mA	10 mA	18 mA	17 mA	
Coagulation	T.	10 min.	12 min.	10 min.	17 min.	12 min.	12 min.	

TABLE 5. The changes of blood pressure, hematocrit, and platelets in electro-coagulation group (12 V) for the uncontrollable bleeding state.

12 V		No.	21	22	23	24	25	Mean
	Sex	F	M	F	M	M		
	Weight	13.0 kg	13.0 kg	8.0 kg	8.0 kg	10.0 kg		
Blood pressure (mmHg)	b.	114	120	120	72	110	108	
	e.	100	122	108	80	122	106	
Ht. (%)	b.	39	42	25	43	53	41	
	e.	43	46	36	38	40	42	
Platelet	b.	147200	176000	142000	113000	142500	156000	
	e.	96000	144000	118000	57000	106500	99000	
Current	Q.	25 mA	17 mA	18 mA	23 mA	37 mA	24 mA	
Coagulation	T.	12 min.	5 min.	21 min.	15 min.	16 min.	14 min.	

cavity were filled with the red blood cell.

Other prominent changes in tissue of the spleen itself were not recognized²⁷⁾ (photos. 8, 9, 10, 11 and 12).

TABLE 6. Average values in the change of blood pressure.

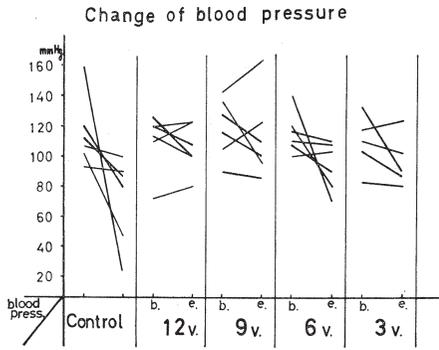


TABLE 7. Average values in the change of hematorit.

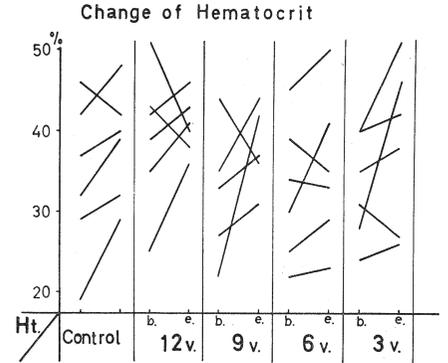


TABLE 8. Average values in the change of platelets.

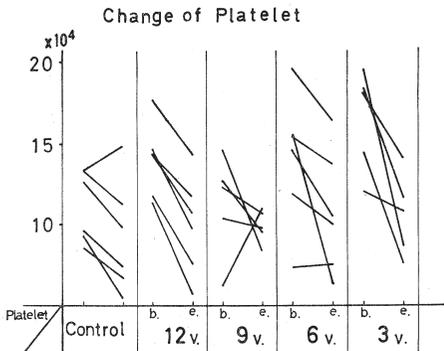
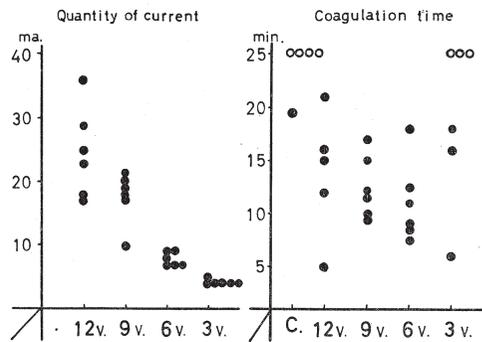


TABLE 9. Quantity of current and duration of time needed to coagulation.



DISCUSSION

The various methods of potential difference measurement by many investigators up to now were by means of electrode inserted directly or indirectly through a branch into vessel of living animals (thoracic and abdominal aorta or venae cavae) and connected to galvanometer. Being effected by so many factors such as surrounding tissue of blood vessel, blood stream itself, and injured current which occurred at the moment of electrode insertion into blood vessel, potential difference measured by many methods previously discussed was not always accurate. So the range of error of potential difference measurement was considerably large.

In 1953, Sawyer⁹⁾ performed the experiments using adult mongrel dogs with sterile technique. The internal electrode was inserted into a branch and

passed through it into the lumen of the main blood vessel. A Sanborn 'Poly Viso' twin channel direct current amplifier was used to make continuous potential difference recordings. The Leed and Northrup potentiometer was used to check results with the Sanborn potentiometer. Three types of electrodes were used to measure the potential difference of the blood vessel wall: 1) calomel cell-catheter electrodes, 2) silver chloride electrodes and 3) flat platinum electrodes. He reported that normal potential difference of grossly undamaged, untransected arteries ranged from -3 to -15 mV with the intima negative.

Fukuta²⁸⁾ achieved the supplemental examination with platinum electrode in 1962, as reference to the Sawyer's experiments which had been performed up to that time. He recognized that potential difference of the normal blood vessel wall was 1 to 10 mV. And the intima was charged negatively with respect to the adventitia. He noted, when the blood vessel was injured that potential difference decreased gradually followed by the reversal of polarity.

In 1957, Eisner¹⁵⁾ recognized also that the reversal of polarity played an important part with regards to intravascular thrombus formation.

When the platinum wire electrode which was used for potential difference measurement of the blood vessel wall, it was found that the platinum wire electrode caused polarization which prevents accurate measurement of potential difference. Therefore, in these experiments, a new apparatus was designed according to Motokawa's²⁹⁾ report, "Apparatus for measurement of potential difference and movement of the removed intestine". The comparative electrode (Model HC-205), non-polarizable electrode, was utilized also.

The reason to use this electrode is that at the contact surface between liquids, ion moves freely and does not accumulate. On the other hand at the contact surface between liquid and metal, ion accumulates easily (phenomena of polarization). When the current from living animal is measured with metallic electrode, it is difficult to catch the micro-current from living body due to phenomena of polarization occurring around the metallic electrode. And so, on account of obtaining the accurate results and preventing such an action of polarization, non polarizable electrode (comparative electrode) was used in this experiment.

Due to prevention of the undesirable effect of potential difference which occurred between liquids, 1 per cent KCl solution and Tyrode solution or Ringer-Lock solution, the glass vessel was filled with 1 per cent KCl solution because the comparative electrode was filled with the saturated solution of KCl. With a comparative electrode, the electric charge across the blood vessel wall and the reversal of polarity in potential difference of the removed blood vessel wall, as described in the reports of other investigators, was obtained.

More accurate values were able to be obtained by this apparatus, when measurements were taken repeatedly. However, if a detailed analysis is made

about such a potential difference, it might be diffusion potential difference which passed through the blood vessel wall.

In the future, when the superior electrode of higher sensitivity and micro-type is developed and is used together with the prebox, the true potential difference across the blood vessel wall may be measured *in vivo*. Also, the attempts to utilize intravascular thrombus formation due to direct current as hemostasis have been carried out by many investigators up to now.

Especially, the electrical hemostasis has been applied to patients directly by Sawyer since 1961. He obtained many successful clinical results, but he did not describe in detail the size of the injured portion, condition of patient during electro-coagulation, blood pressure, hematocrit, and source of electricity.

Comparing to alternating current electro-coagulation, direct current electrical hemostasis is superior in that it shows no significant damage histologically and morphologically, though it takes quite a long time to obtain complete coagulation because of the small current.

It was found from these experiments that the application of this method in the clinical field is effective in uncontrollable bleeding in injured organs, as previously reported by Sawyer^{16),20),21),22)} (1961) and Kravitz²⁴⁾ (1964).

In clinical application, if positive electrode mesh knitted by platinum wire, 0.2 to 0.5 cm in diameter, is made if injured organ is wrapped completely by the platinum-mesh, if the application of a positive current is carried out regularly at the rate of 1 to 2 times a day, more successful hemostatic result may be obtained³⁰⁾.

On the present microscopic observation of intravascular thrombus formation by direct current, the positive electrode was placed near a capillary which was selected indiscreetly in the visual field. The electrode should be inserted into the capillary or made contact with the blood vessel wall, but this technique is very difficult.

However, the individual movement of platelets or other kind of blood cells, the important factors which are directly concerned with hemostasis, was not observed in detail.

It is very interesting to find that there is a relationship between hemostatics or anticoagulants and electro-coagulation when investigated with the microscope. In the future, such records of magnified observation of the dynamic changes of the thrombus formation could be recorded by high-speed movie, too.

SUMMARY

(1) The measurement of potential difference across the removed blood vessel wall of dog's abdominal aorta, *in vitro*, was carried out with the newly developed apparatus and the superior comparative electrode.

The pure potential difference was measured successfully by this apparatus

under stable condition.

(2) The process of intravascular thrombus formation due to 6 V, 4 to 4.5 mA positive current being applied upon the mesenterial capillary of rabbit was observed microscopically.

(3) The electro-coagulation for uncontrollable bleeding from the injured portion of a definite size on the spleen of dog was carried out. Its effect was investigated, and the changes of blood pressure, platelets, and hematocrit of the experimental animals was observed. And it was recognized that the direct current coagulation is very effective on uncontrollable bleeding from ulcerbed or injured organ in which ligation or suturing can not be done.

ACKNOWLEDGEMENT

The author would like to give his deep gratitude to Prof. Dr. Y. Hashimoto for his kind guidance throughout this study, and to Assist. Prof. Dr. K. Kamiya and K. Iwaya M. D. for their kind help with the procedure in this investigation.

Apart of this paper was presented at the 4th Congress of Japanese Society for Artificial Organ and Tissues in 1966.

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EXPLANATION OF PHOTO.

- PHOTO. 1. The normal blood stream in the capillary before the imposition of a positive current.
- PHOTO. 2. After 6 V, 1 to 1.5 mA of positive current were imposed for 1 to 2 minutes, sludge phenomena appeared in a part of capillary.
- PHOTO. 3. After 2.5 minutes, sludge disappeared and normal blood stream in capillary returned.
- PHOTO. 4. After 5 to 6 minutes, sludge appeared again in various places. The blood stream in capillary became slowly.
- PHOTO. 5. After 5 to 6 minutes, sludge appeared again in various places. The blood stream in capillary became slowly.
- PHOTO. 6. After 12 minutes, the blood stream stoped completely, and thrombus formation began.
- PHOTO. 7. After 13 to 14 minutes, all capillaries changed to the homogeneous state, and thrombus formation was completed.
- PHOTO. 8. Histological picture in the completion of coagulation (control). Hematoxylin and eosin. $\times 200$
- PHOTO. 9. Histological picture in the completion of electro-coagulation with the imposition of 3 V positive current. Hematoxylin and eosin. $\times 200$
- PHOTO. 10. Histological picture in the completion of electro-coagulation with the imposition of 6 V positive current. Heamtoxylin and eosin. $\times 200$
- PHOTO. 11. Histological picture in the completion of electro-coagulation with the imposition of 9 V positive current. Hematoxylin and eosin. $\times 200$
- PHOTO. 12. Histological picture in the completion of electro-coagulation with the imposition of 12 V positive current. Hematoxylin and eosin. $\times 200$

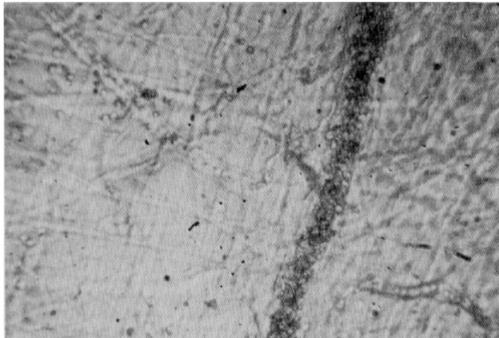


PHOTO. 1

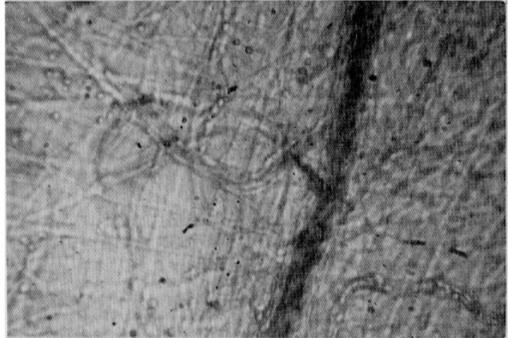


PHOTO. 2

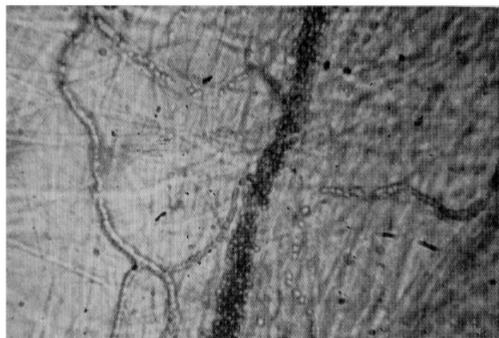


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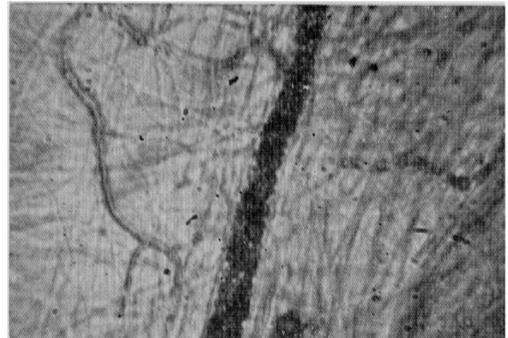


PHOTO. 4



PHOTO. 5

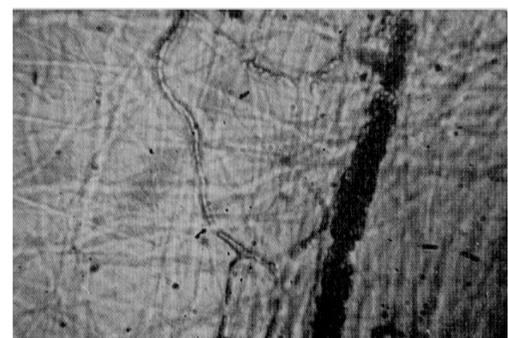


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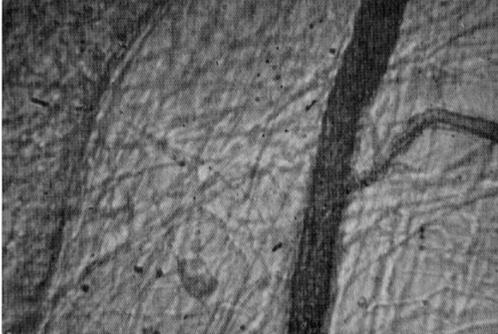


PHOTO. 7



PHOTO. 8



PHOTO. 9

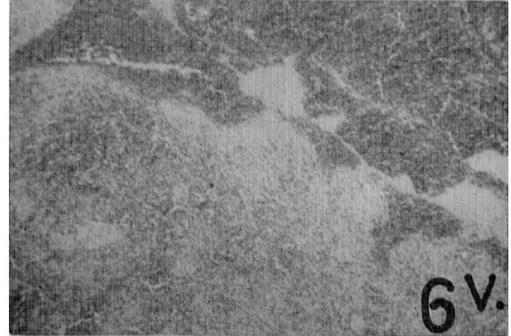


PHOTO. 10



PHOTO. 11



PHOTO. 12